30.6 A Wireless Bio-MEMS Sensor for C-Reactive Protein Detection Based on Nanomechanics

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Recently, detection of DNA and proteins based on the bending of cantilevers has been demonstrated [1-3]. However, their cantilevers can only be reused by dissociating the DNA/proteins with strong chemical bases/acids, which inevitably handicaps their use in portable/wearable applications. Previously, it was found [4] that nonspecific immunologic disease-related proteins can be removed from the cantilever with electrical manipulation. In this paper, we further demonstrate that cardiovascular-event-related proteins, such as CRP [5], can also be dissociated by applying a low frequency ac electric field to the sensor. This allows the design of a wireless label-free detection system for disease-related C-reactive proteins (CRP) using a microcantilever with a safe reusable feature.

The CRP concentration in human serum is below 1ug/mL for a healthy person but may rise up to 100 or even 500 times in response to infection in the body. Recent studies have also shown that high levels of CRP in the bloodstream raise the risk of a heart attack [5]. Hence wireless wearable biosensors are desirable because monitoring users' health in their normal living situation significantly improves their quality of life.

The CRP sensing mechanism is illustrated in Fig. 30.6.1. First, CMOS compatible silicon nitride was deposited on the silicon substrate. Then a MEMS cantilever was fabricated by photolithography followed by micromachining technology. Optimum sensor size of a length of 200 um and each leg 40 um wide was determined by the balance among spring constant, bio-induced stress and stability in the flow field. On the top side of the cantilever, chromium, gold, biolinker, and anti-CRP were deposited. Chromium was adopted to improve the adhesion of gold to silicon nitride while the gold layer was used to immobilize the biolinker. The anti-CRP was bonded to the biolinker for probing CRP. Specific biomolecular interactions between CRP and anti-CRP alter the intermolecular nanomechanical interactions within the biolinker layer. As a result, the cantilever is bent. The bending of the cantilever can be measured by optical beam deflection or piezoresisive techniques. In this paper, the former is adopted because of its excellent minimum detection limit of 0.1nm.

The experimental setup is also shown in Fig. 30.6.1. After placing a poly-dimethyl-siloxane (PDMS)-based "cap" above the functionalized cantilever, two microfluidic channels and one liquid chamber for reaction were formed. The reagents were injected into the channels and chamber using a syringe pump. A laser beam through the optically transparent PDMS cap was focused onto the tip of the cantilever with the help of a CCD camera and hence the alignment among the laser beam, the cantilever and the position-sensitive detector (PSD) was insured. The function blocks of the wireless CRP measurement system are illustrated in Figs. 30.6.2 and 30.6.3. Custom commands from a personal computer were received by a novel 0.45V operated ASK receiver consisting of a common-source amplifier, gain stages, and a diode-RC demodulator. The 0.45V operation is achieved by using low threshold (0.2V) transistors. After decoding the commands, the MCU activated the 8b charge-redistributed ADC and preamplifier to convert the analog bio-signals measured by the commercially available PSD into digital values. Then an ASK transmitter comprising a ring oscillator and a common-source class-C power amplifier transmitted the digital values to the PC. Figure 30.6.4 summarizes the measured performance of the wireless ASK circuit.

The measured deflection as a function of time with various CRP concentrations is shown in Fig. 30.6.5. The concentration data shown are highly correlated (R=0.99956) against concentrations achieved via turbidimetric immunoassay. Specificity and no false positive were confirmed by the negligible deflections of the non-functionalized cantilever with the injection of 100ug/mL CRP and the functionalized cantilever with the injection of bovine serum albumin (BSA). Clearly, deflection due to specific CRP binding can be detected for CRP concentrations from 1ug/mL to 500ug/mL, covering the clinically relevant range. The CRP concentration can be determined at any arbitrary time depending on the accuracy required. A longer time yield better accuracy since the noise is approximately a constant (~5nm) but a shorter time (<30 minutes) is sufficient because very accurate CRP level is not required for clinical applications. Different slopes at the origin can also be used as a criterion. From Fig. 30.6.5, the achievable dynamic range is estimated to be 250nm which is limited by the total deposited amount of anti-CRP. Note that after binding, the CRP can be physically unbound from the cantilever by applying a low frequency (0.2 Hz) ac electric voltage (1V) signal between the gold electrode connected to the cantilever and nickel electrode around the cantilever as indicated in Fig. 30.6.1. Figure 30.6.6 shows the temporal response of the cantilever before and after applying the ac electrical signal. The cantilever was successfully brought back to its initial position after the application of the ac electric signal and the same cantilever was reused for successive experiments. No noticeable aging effect was found i.e. the CRPs still could be completely dissociated from the cantilever at least after 6 cycles. This physical unbinding method is in contrast to the traditional methods using bases/acids [1] [2] [3]. Since [1] reported that the cantilever can be reused for at least 10 times, it is believed that our "mild" method should yield a longer lifetime.

Although the He-Ne laser, the reflected mirror and the lens were used here for preliminary demonstration, it should be noted that semiconductor lasers [1] [3] and microlenses used in a compact disk (CD) player can be placed on top of the PDMS cap to fulfill the purpose equally well. That is, a wireless bio-MEMS sensor with the size of a CD player can be expected. Furthermore, the silicon nitride passivation layer used in standard CMOS technology can be used as the cantilever after post circuit processing and thus the bio-MEMS sensor can be integrated with the wireless circuit on the same die. For further size shrinkage, even the standard CMOS poly-resistor layer can be used as the cantilever for the detection of the piezoresistive change due to bending.

Acknowledgments:
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[4] R.-Z Hwang et al., "A Novel Reusable Nanomechanics-Based Protein Biosensor with Electrical Manipulation," *IEEE MEMS*, pp. 798-801, 2005
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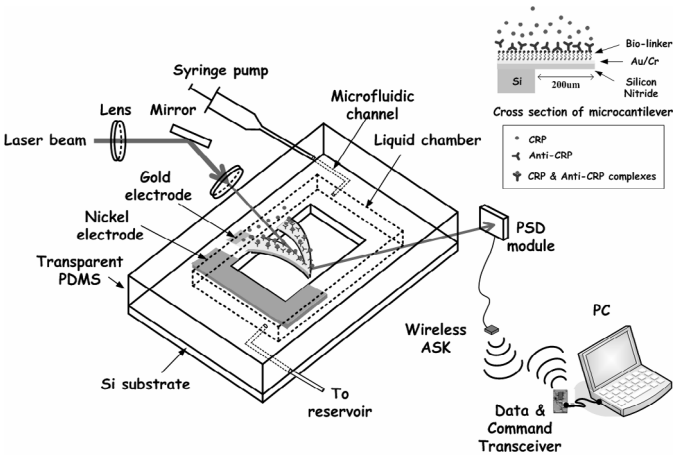

Figure 30.6.1: CRP sensing mechanism and experimental setup.

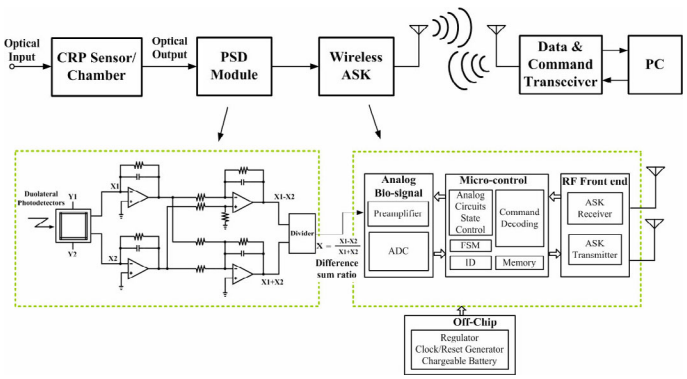

Figure 30.6.2: Function blocks of the wireless CRP measurement system.

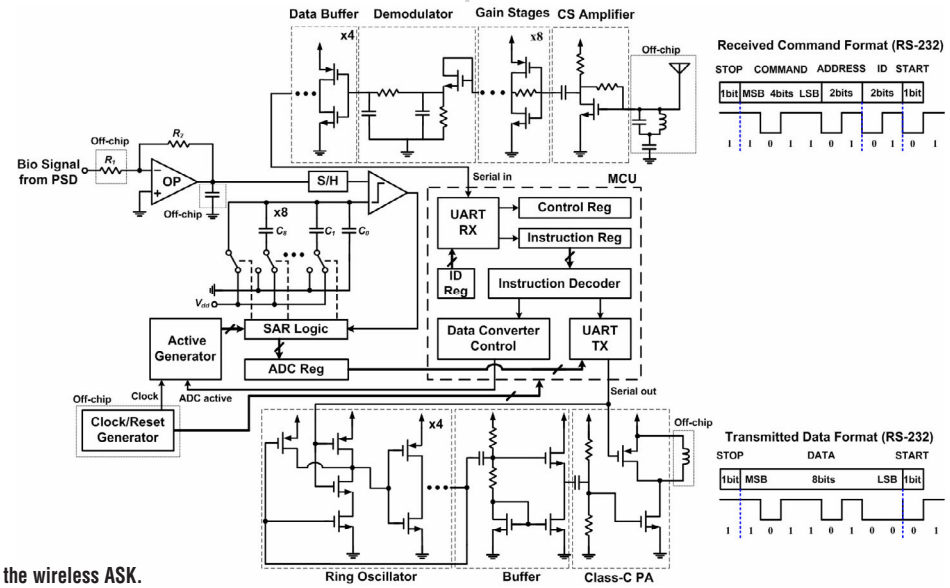

Figure 30.6.3: Schematic of the wireless ASK.

Technology	0.18μm CMOS
Chip area	3.4mm²
Preamplifier AC gain	0~60dB
Micro-control unit operation clock	500kHz
8bits charge-redistributed ADC	
Clock rate	500kHz
Conversion rate	25 kSamples/s
INL/DNL	±1 LSB/ ±0.5 LSB
SNDR	47dB
ENOB	7.5 bits
Power consumption	135μW @ 1.8V
ASK transmitter	
Carrier frequency	433.9 MHz
Maximum data rate	500kb/s
Output power	-20 dBm @ 50Ω
Power consumption	19mW @ 1.73V
ASK receiver	
Sensitivity	<-35 dBm @ 433MHz
Max data rate	~2Mb/s
Power consumption	1.36mW @ 0.45V

Figure 30.6.4: Measured wireless ASK performance summary.

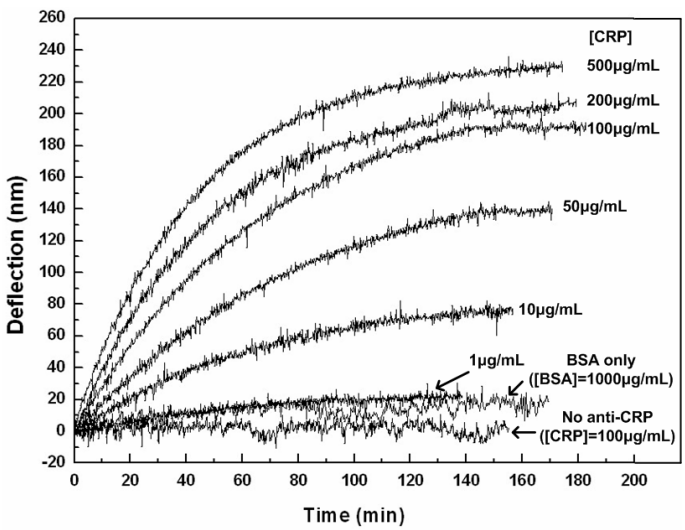

Figure 30.6.5: Measured deflection as a function of time with various concentrations of CRP.

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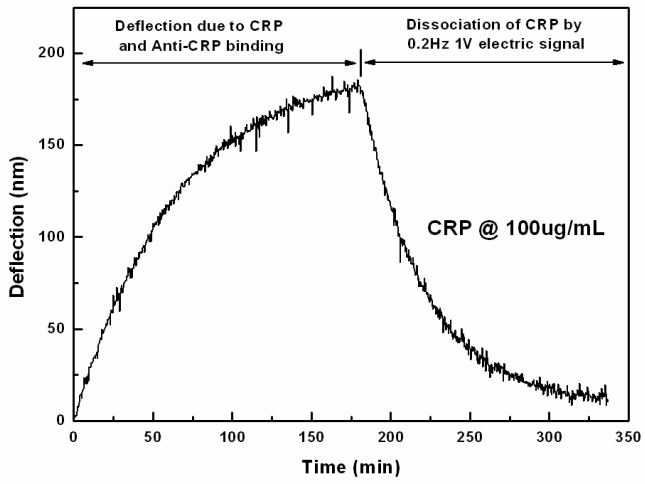
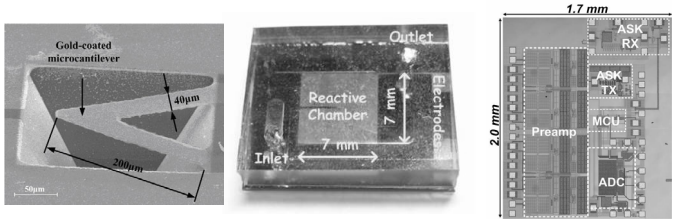


Figure 30.6.6: Temporal response of the microcantilever.



Microcantilever Reaction chamber Wireless ASK circuit

Figure 30.6.7: Chip photos.